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Enhancement of Hemoglobin and F-Cell Production by Targeting Growth Inhibition and Differentiation of K562 Cells With Ribonucleotide Reductase Inhibitors (Didox and Trimidox) in Combination With Streptozotocin

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Upon appropriate drug treatment, the human erythroleukemic K562 cells have been shown to produce hemoglobin and F-cells. Fetal hemoglobin (Hb F) inhibits the polymerization events of sickle hemoglobin (Hb S), thereby ameliorating the clinical symptoms of sickle cell disease. Ribonucleotide reductase inhibitors (RRIs) have been shown to inhibit the growth of myeloid leukemia cells leading to the production of Hb F upon differentiation. Of the RRIs currently in use, hydroxyurea is the most effective agent for Hb F induction. We have examined the capacity of two novel RRIs, didox (DI) and trimidox (TRI), in combination with streptozotocin (STZ), to induce hemoglobin and F-cell production. The K562 cells were cultured with different concentrations of didox-STZ or trimidox-STZ at a fixed molar ratio of 3:1 and 1:5 for 96 hr, respectively. At pre-determined time intervals, aliquots of cells were obtained and total hemoglobin (benzidine positive) levels, number of F-cells, and Hb F were determined by the differential staining technique, fetal hemoglobin assay kit, and fluorescence cytometry respectively. The effect of combined drug treatment on the growth of K562 cells was examined by isobologram analysis. Our results indicate that a synergistic growth-inhibitory differentiation effect occurred when didox or trimidox was used in combination with STZ on K562 cells. There was an increase in the number of both benzidine-positive normoblasts and F-cells, accompanied by morphologic appearances typical of erythroid maturation. On day 4, the number of benzidine-positive cells showed a 6–9-fold increase and the number of F-cells was between 2.5- and 5.7-fold higher than the respective controls. Based upon these results, treatment with a ribonucleotide reductase inhibitor, such as didox or trimidox, in combination with STZ, might offer an additional promising option in sickle cell disease therapy. Am. J. Hematol. 63:176–183, 2000.

Key words: ribonucleotide reductase inhibitors; sickle cell anemia; erythroleukemia cells

INTRODUCTION

Sickling of red blood cells in patients with sickle cell anemia is caused by the formation of insoluble and rigid polymers of sickle hemoglobin (Hb S) under hypoxic conditions. The intracellular level of fetal hemoglobin (Hb F) has a major clinical impact in patients with β-hemoglobinopathies (sickle cell anemia and β-thalassemia). Epidemiological studies on sickle cell anemia have indicated that an increase in Hb F level ameliorates the clinical symptoms of the underlying disease. Not only do

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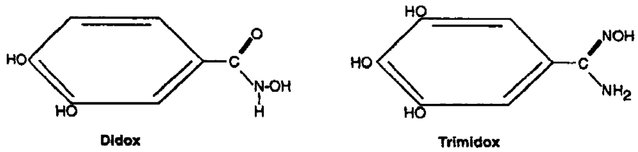

Fig. 1. Chemical structure of didox and trimidox.

Hb F-containing cells have a lower concentration of Hb S, but Hb F also directly inhibits polymerization, which accounts for the lower propensity of such cells to form intracellular polymers and undergo sickling [1,2].

Induction of Hb F has therefore been considered an effective approach in therapies for sickle cell disease (SCD). Several pharmacologic agents including hydroxyurea [3,4], 5-azacytidine [5], and sodium butyrate [6] have been shown to increase the Hb F level in SCD patients. The limitations of some of these agents include their cytotoxicity as well as their less than optimum induction of Hb F.

Hydroxyurea (HU), a relatively weak inhibitor of ribonucleotide reductase [7], is the only drug in clinical use for the management of sickle cell disease. Therefore, it is important to test the effect of combined treatment of effective agents that have different mechanisms of action. Didox (3,4-dihydoxybenzohydroxamic acid) and trimidox (3,4,5-trihydroxybenzamidoxime) (Fig. 1) are inhibitors of ribonucleotide reductase and inhibit the growth of tumor cells [8–10].

In vitro and in vivo studies have indicated that didox and trimidox induce fetal hemoglobin production [11,12]. In their in vivo studies, Pace et al. observed that administration of didox to transgenic sickle cell mice and an anemic baboon resulted in high level of γ-globin gene expression [11]. Streptozotocin, on the other hand, is an anti-neoplastic agent that has been in use in combination regimens in cancer chemotherapy [13,14]. One of the unique pharmacological properties of STZ is its antitumor activity without bone marrow toxicity [15]. Recently, we found that STZ has the capacity to induce Hb F synthesis in human erythroleukemia cells (unpublished data). Pre-clinical evaluation of therapeutic drugs for possible synergistic interactions is important since it can lead to the development of clinical trials with rational combinations of drugs based on models of drug synergy [16]. Therefore, it is important to test more effective induction of Hb F than that which is currently achieved by single-agent therapy by combining different agents, to result in more efficacious therapy. In this study, we examined whether a combination of didox or trimidox (more effective ribonucleotide reductase inhibitors than HU) with STZ exhibits a synergistic effect on the differentiation of human erythroleukemic K562 cells, accompanied by enhanced production of hemoglobin and F-cells.

MATERIALS AND METHODS

Drugs

Didox and trimidox were synthesized as described previously [10,17], and were a gift from Dr. van't Riet of Molecules for Health Inc., Richmond, VA. Streptozotocin was purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Culture and Reagents

The human erythroleukemia K562 cell line, which was purchased from ATCC (Manassas, VA), was maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS) (Atlanta Biologicals), and 50 U/mL of streptomycin–penicillin. The cultures were maintained in 5% CO_2 atmosphere and cell viability was greater than 92% as determined by trypan blue exclusion. The Giemsa reagent, fetal hemoglobin kit, trypan blue solution, and hydrogen peroxide were all purchased from Sigma. The acid hematoxylin was obtained from Fischer Scientific (Pittsburgh, PA).

Dose–Response Studies

In this assay, K562 cells (1×10^5 cells/mL) were seeded in 25-cm^3 flasks and incubated in RPMI 1640 medium (37°C) containing various concentrations of didox, trimidox, or STZ (2–100 μM) for 96 hr. Cell number was counted by trypan blue exclusion assay. Each condition was set at triplicate. The IC_{50} value (concentration of drug that inhibits half the cell population) for each compound was calculated using the method of Chou and Talalay [18].

Time Course Studies

The K562 cells (1×10^5 cells/mL) were incubated in RPMI 1640 medium supplemented with 10% FBS in the presence of 23 μM didox, 7 μM trimidox, or 16 μM STZ at 37°C for 4 days. At pre-determined time intervals, an aliquot of cells was collected for growth inhibition studies. Cell viability was determined by trypan blue exclusion. A parallel control experiment without the addition of drugs was simultaneously performed. The above drug concentrations were used in this assay because preliminary studies showed that these concentrations optimally increased the total hemoglobin level and F-cell production.

Effect of Drug on Cell Morphology

The K562 cells were seeded in 25-cm^3 flasks at a density of 1×10^5 cells/mL and incubated in culture with 23 μM didox, 7 μM trimidox, or 16 μM STZ at 37°C for 96 hr. At the end of the incubation period, 2.0 mL of the incubated cell suspension were collected and washed with 4 mL phosphate buffer saline (PBS) by centrifugation at 2,300 rpm for 3 min, followed by reconstitution of

the cell pellet in 2.0 mL of PBS. Finally, 50 μL of the cell suspension was transferred to a microcentrifuge tube and centrifuged at 2,000 rpm for 2 min in a cytocentrifuge. The cells were fixed in methanol for 5 min and air-dried, followed by differential staining with Giemsa stain (diluted 1:20 with deionized H_2O) for 30 min. The cells were air-dried, mounted, and viewed under a microscope.

Upon treatment of cells with Giemsa reagent, the cell cytoplasm assumes a characteristic pink coloration, and the cell morphology was easily visualized. Changes in cell morphology were assessed based on cell sizes. Control cells were large, whereas drug-treated cells were generally of intermediate and small sizes.

Effect of Combined Drug Treatment on Benzidine-Positive Cells

The number of benzidine-positive cells (total hemoglobin) was determined using the modified method of McLeod et al. [19]. Benzidine-positive cells stained bright orange, whereas negative cells stained blue. The K562 cells were seeded in 25-cm^3 flasks at a density of 1×10^6 cells/mL and incubated in culture with a mixture of 2 μM trimidox and 10 μM STZ (1:5), or a mixture of 12 μM didox and 4 μM STZ (3:1), for 96 hr. At 24-hr intervals, 2.0 mL of the incubated cell suspension was collected and washed with an equal volume of PBS followed by centrifugation at 2,300 rpm for 3 min. The supernatant was aspirated and the cell pellet was reconstituted in 2.0 mL PBS. Finally, 50 μL of the cell suspension was transferred into a microcentrifuge tube and centrifuged at 2,000 rpm for 2 min in a cytocentrifuge. The cell pellet was further treated as follows: The slide containing the cell pellet was immersed in benzidine (1% benzidine in methanol) for 4 min. It was then immediately transferred into a H_2O_2 (2.5% H_2O_2 in 70% ethanol) solution for 1 min. The treated cell pellet was rinsed in running water and dried with a dryer, followed by a further treatment with hematoxylin for 15 sec. After being rinsed with running water and drying with a dryer, the slide was mounted (with coverslip) under the microscope. The benzidine-positive cells (brown to orange) were scored and expressed as a percentage of the number of total cells. The control experiment was simultaneously performed without addition of drugs. After testing different drug combinations, the above drug combinations of didox-STZ and trimidox-STZ resulted in maximum induction of total hemoglobin and F-cell production as well as effective growth inhibition without concomitant cytotoxicity (cell viability > 92%).

Effect of Combined Drug Treatment on the Induction of F-Cell Production

To determine whether the combined drug concentrations stimulate the induction of F-cell production in K562 cells, we treated the cells (1×10^6 cells/mL) in RPMI-1640 medium with a mixture of trimidox and STZ at a molar ratio of 1:5, or didox and STZ at a molar ratio of 3:1 at 37°C for 4 days. At pre-determined time intervals, an aliquot of cells was collected and the number of F-cells was determined with an assay kit according to the supplier's instructions.

Briefly, a 10 × dilution of the supplied citrate phosphate buffer solution was prepared fresh daily and prewarmed at 37°C before use. At 24-hr intervals, 2.0 mL of the incubated cell suspension was collected and washed twice with 4.0 mL of PBS. Subsequently, 50 μL of the washed cell suspension was transferred into micro-tube and centrifuged at 2,000 rpm for 2 min using the cytocentrifuge.

Slides were fixed by immersion in an ethanol fixative for 5 min and then rinsed thoroughly with tap water and air-dried. Thereafter, the slides were immersed in prewarmed citrate phosphate buffer for a total of 5 min, followed by agitation after immersions for 1 and 3 min. The slides were then stained in acid hematoxylin solution for 3 min and rinsed with distilled water. Excess water was removed by shaking, and the slides were counterstained in 0.1% Eosin B solution, followed by thorough rinsing with distilled water and air-drying. Finally, slides were examined using an oil immersion microscope. Cells retaining Hb F (F-cells) were scored and expressed as a percentage of the total number of cells. Retained Hb F causes cells to appear red and the presence of ghost cells indicates the absence of fetal hemoglobin.

Quantitation of Fetal Hemoglobin Level by Fluorescence Image Cytometry

The staining of cells containing Hb F and its quantitation were performed as previously described [20–22] with slight modification. Briefly, 1×10^5 cells/mL were incubated in culture with a mixture of STZ and didox or trimidox for 96 hr. At various time intervals, an aliquot of cells was collected and fixed with a solution consisting of acetone, methanol, and ethanol (3:1:1), followed by washing with phosphate buffer saline (PBS) for 10 min. The sample was then air-dried, and the smear was incubated with fetal bovine serum (FBS) for 30 min at 37°C in a humidified chamber.

The sample was then stained for Hb F with a monoclonal antibody (specific for γ-globin gene) conjugated with fluorescence probe, FITC (Wallac Inc., Akron, OH) for 30 min at 37°C in a humidified chamber. The specimen was then washed with PBS and distilled water for 10 min, followed by air-drying for another 10 min. Antifading gel was applied on the stained area in order to minimize fluorescence bleaching during the image acquisition [21]. Under fluorescence microscopy, cells containing Hb F appear green whereas, cells without Hb F are invisible. The color images were obtained by color

cooled CCD camera (C5810, Hamamatsu Photonics K.K., Hamamatsu, Japan), and quantitated by its software.

Analysis of Combined Drug Effect

The effect of combined drug treatment on K562 cells was examined. The dose effect was determined for each compound alone and at a fixed molar ratio with the second compound. The interaction of the two compounds was quantified by determining a combination index (CI) value according to the method described by Chou and Talalay [18]:

$$CI = (D)_1/(D_x)_1 + (D)_2/(D_x)_2 + (D)_1(D)_2/(D_x)_1(D_x)_2,$$

where D_x represents the minimum dose of each drug alone that produces an effect, while $(D)_1$ and $(D)_2$ are the doses of drugs 1 and 2 in the mixture that produce the same effect. This analysis generates the combination effect as follows: summation (additive or zero interaction) is indicated when CI = 1; synergism is indicated when CI < 1; antagonism is indicated when CI > 1.

Statistical Analysis

The data were analyzed statistically using the Instat program (Graph Pad Software, San Diego, CA). The standard error bars are as shown in the graphs.

RESULTS

Effect of Drugs on Growth Kinetics of K562 Cells

Human erythroleukemia K562 cells were incubated with various concentrations of trimidox, Didox, and streptozotocin for 4 days. Trimidox exhibited an IC_{50} concentration of 7 μM, whereas didox and STZ inhibited cell proliferation with an IC_{50} concentration of 23 and 16 μM, respectively . The results of the growth kinetics of K562 cells upon treatment with trimidox, didox, or STZ are shown in Figure 2. As shown in Figures 2 and 3, trimidox, didox, and STZ inhibit the growth of K562 cells in a concentration- and time-dependent manner. The effect of drugs on cell growth was accompanied with morphological changes typical of erythroid differentiation. During the first 2 days of culture, there was no observed change in the doubling time of 24 hr. Treatment of cells with trimidox, didox, or STZ appeared to cause an arrest of growth at 24 hr. The doubling time in response to the RRIs and STZ was augmented to approximately 36 and 48 hr, respectively.

To examine whether STZ is synergistic with didox or trimidox, cells were incubated with increasing concentrations of STZ and didox or trimidox at a fixed molar ratio, and the effect on cell growth was determined. The effect of different molar ratios of drugs was also examined. As shown in Table I, the combination of STZ with didox (1:3 molar ratio) or STZ with trimidox (5:1 molar ratio) inhibited the growth of K562 cells to a greater degree than either agent alone. Analysis of the combined effect of these drugs on cell proliferation by the method of Chou and Talalay revealed that their effects were synergistic.

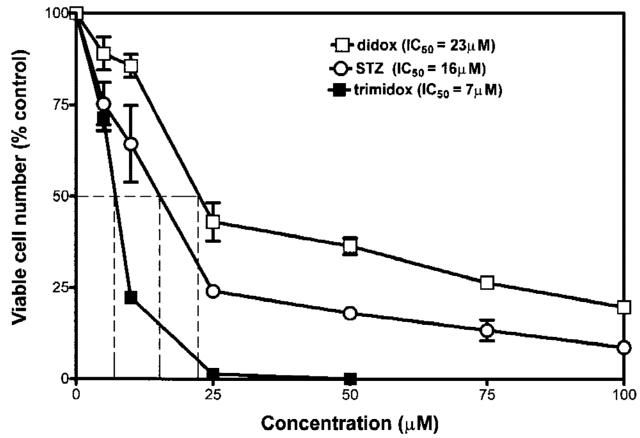

Fig. 2. Growth inhibitory effect of didox, trimidox, and STZ on K562 cells. The K562 cells were incubated in the presence or absence of various concentrations of didox, trimidox or STZ for 4 days. Cell viability (>92%) and growth inhibition were determined by trypan blue exclusion assay. The mean ± SEM of triplicate assays is given, plotted as the percentage of respective control values. The vertical (broken) lines indicate the IC_{50} concentrations of the individual drug.

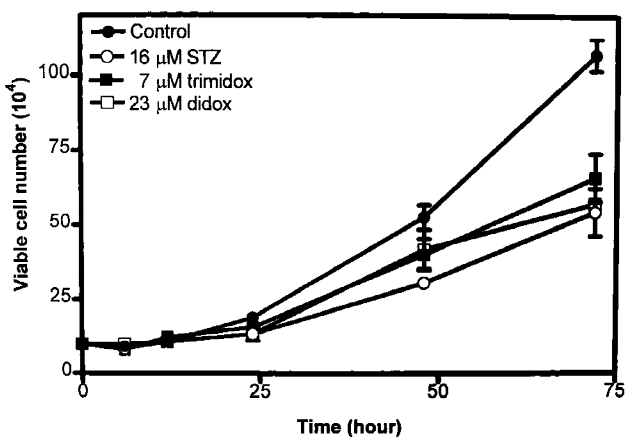

Fig. 3. Time course of the inhibition of K562 cell growth by didox, trimidox, and STZ. Cells were cultured with didox, trimidox, or STZ for 4 days. At time intervals, an aliquot of cells was collected to determine the extent of cell inhibition, using the trypan blue exclusion assay as detailed in materials and methods. The means ± SEM of triplicate assay are given and plotted as total viable cell number.

Combined Drug Treatment on Hemoglobin and F-Cell Production in K562 Cell Line

To study the combined effects of trimidox and STZ or didox and STZ on hemoglobin and F-cell production in

TABLE I. Growth Inhibitory Effect of Didox, Trimidox, and STZ on K562 Cells†

Agents	Concentration (μM)	Cell no. (% control)	Predicted value	Combination index
(i) Didox (A)	18	67		
	30	41		
	120	19		
STZ (B)	6	72		
	10	68		
	40	22		
Didox + STZ	18			
	6	48	48	0.97*
Didox + STZ	30			
	10	38	28	0.86*
Didox + STZ	120			
	40	5	4	0.49*
(ii) Trimidox (A)	3	74		
	5	61		
	10	20		
STZ (B)	15	51		
	25	25		
	50	18		
Trimidox + STZ	3			
	15	27	38	0.90*
Trimidox + STZ	5			
	25	18	15	0.70*
Trimidox + STZ	10			
	50	3	4	0.50*

†The K562 cells were incubated in culture medium and supplement with didox or trimidox and STZ for 3 days. The effect on cell growth was determined as described in Materials and Methods section. Data are means of at least three determinations, and standard error (SEM) values were within 5%. Predicted values were calculated as follows: ($A \times B$)/100 (%). Combination index was determined as described by Chou and Talalay [18]. *Synergism: Combination index <1. The values shown are for mutually non-exclusive effects of the two drugs. The values for mutually exclusive effects were slightly lower.

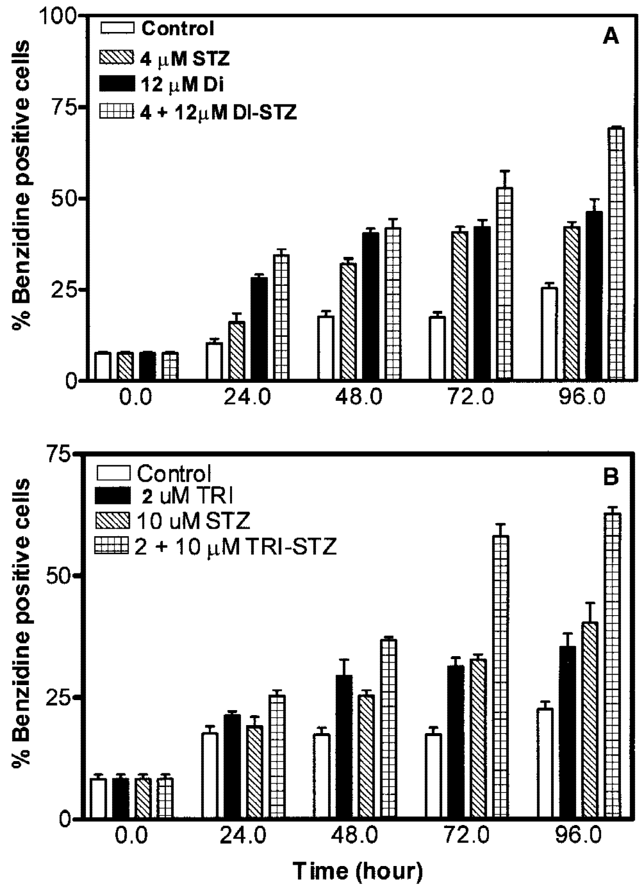

Fig. 4. Effect of combined drug treatment on hemoglobin production in K562 cells. Cells were cultured with didox (A) or trimidox (B) in combination with STZ for 4 days. At 24-hr intervals, an aliquot of cells was collected and the number of benzidine positive cells was determined as described in Materials and Methods. The mean ± SEM of triplicate assay is given and plotted as a percentage of total cell value.

vitro, human erythroleukemia K562 cells were incubated with mixtures of both compounds at a constant molar ratio. The number of benzidine positive cells (hemoglobin-containing cells) was determined using the modified method of McLeod et al. as described in Materials and Methods [19]. After cell treatment, the F-cells (Hb F-containing cells) were determined with an Eosin assay kit and staining procedure. Treatment of cells with didox-STZ, and trimidox-STZ resulted in a significant increase in benzidine positive normoblasts as well as an increase in the number of F-cells as indicated by Hb F-containing cells. Benzidine-positive cells became evident after incubation for 24 hr. At 72 and 96 hr, the mean number of benzidine-positive cells increased 6.8- and 9.0-fold in didox-STZ-treated cells (Fig. 4A) or 6.9- and 7.5-fold among the trimidox-STZ-treated cells (Fig. 4B), respectively. There was also augmentation in the number of F-cells by 3.6-, 6.1-, and 5.7-fold among the didox-STZ-treated cells on day 2, 3, and 4, respectively (Fig. 5A), and by 2.5-, 2.8-, and 3.4-fold among the trimidox-STZ treated cells on day 2, 3, and 4, respectively (Fig. 5B). The combination of STZ with either didox or trimidox enhanced the production of hemoglobin and F-cell production to a greater degree than either drug alone. There was a significant difference in the number of benzidine-positive cells and F-cells between the treated cells and control culture at every time point. Figure 6 represents the fold increase of HbF production among the stimulated F-cells as revealed by fluorescence cytometry. Although the combination of didox or trimidox with STZ elicited comparable induction of benzidine-positive cells, didox appears to be a more effective inducer of F-cell production than trimidox when used in combination with streptozotocin. As shown in Figure 6, there was a 4- and 7 -fold induction of Hb F in trimidox-STZ and didox-STZ–treated sample, respectively, as revealed by fluorescence cytometry. The presence of γ-globin chain was conclusively established by reversed phased HPLC as reported previously [23]. Figure 7 shows the morphological appearances of K562 cells in

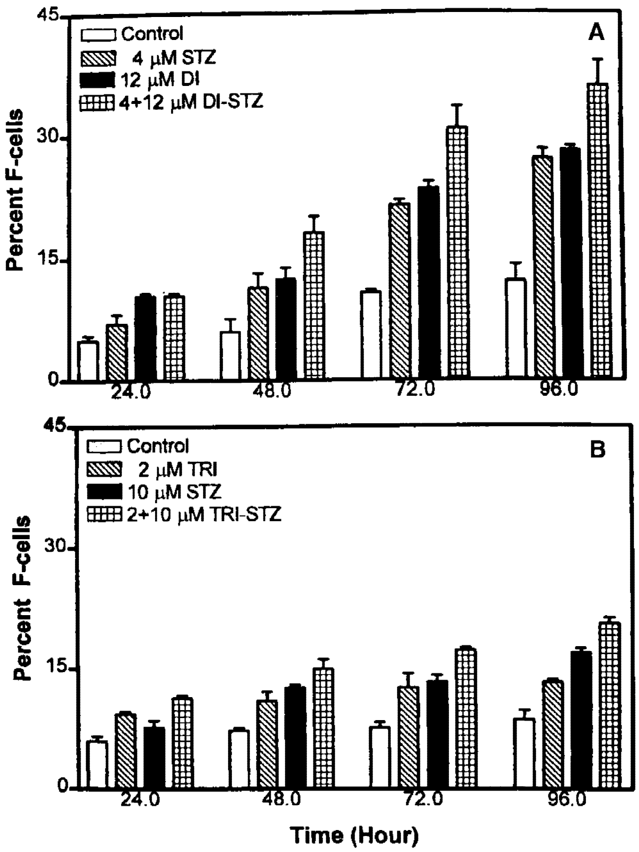

Fig. 5. Effect of combined drug treatment on F-cell production in K562 cells. Cells were cultured with didox (A) or trimidox (B) in combination with STZ for 4 days. At 24-hr intervals, an aliquot of cells was collected and the number of F-cells determined as described in Materials and Methods. The mean ± SEM of triplicate assay is given and plotted as a percentage of total cell value.

control (panel A), trimidox-STZ (panel B), and didox-STZ-treated cells (panel C).

DISCUSSION

Didox and trimidox are polyhydroxy-substituted benzoic acid derivatives, which have been shown to be effective inhibitors of ribonucleotide reductase, a rate limiting enzyme in the de novo synthesis of deoxynucleotides and therefore DNA. These novel ribonucleotide reductase inhibitors have been proven to be effective against tumor cell growth both in vivo and in vitro [24]. Their IC_{50} values are significantly lower than that of hydroxyurea, the only ribonucleotide reductase inhibitor in clinical use for the treatment and management of sickle cell disease [3,4]. Hydroxyurea therapy has resulted in definite improvement in sickle cell patients in terms of reduction of the number of painful crises, but the long-term effects of HU treatment including possible leukemogenesis are presently unclear [25]. The main limitations of HU are myelossuppressive activity, weak ribonucleotide reductase activity, lack of chemical stability, and rapid plasma clearance [26,27].

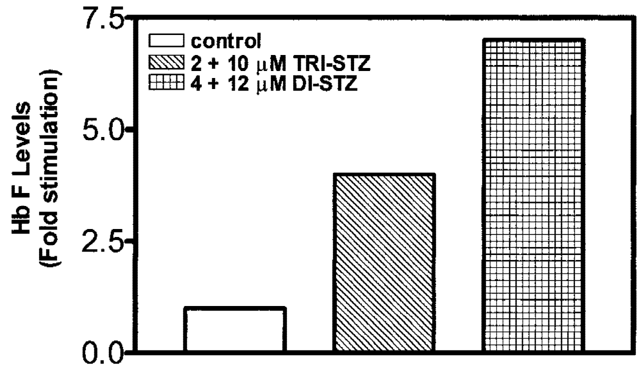

Fig. 6. Effect of combined drug treatment on Hb F production in K562 cells. Cells were cultured and treated with trimidox-STZ or didox-STZ for 4 days, followed by treatment with the monoclonal antibody specific for γ-globin gene as described in Materials and Methods. At 48-hr intervals, an aliquot of cells was collected and the Hb F level was determined as described in Materials and Methods. The fold stimulation of Hb F with respect to the control value is shown.

Most significantly, HU has been shown to be effective in only 60 % of sickle cell disease patients [28,29]. Further, the damaging effects of HU on proteins have been shown to involve the induction of free radical reactions [30]. Although there is evidence that didox and trimidox are mildly myelosuppressive, these compounds have been shown to be excellent free radical scavengers as well as having the capacity to complex iron [24]. In addition to its free radical activity, trimidox has been shown to prevent ischemia reperfusion injury in an isolated rabbit heart model [31]. Streptozotocin, on the other hand, is a non-myelosuppressive agent with potent antitumor activity against islet carcinomas and malignant carcinoids [13]. In view of frequent blood transfusions in individuals with severe sickle cell disease, iron overload has been a major concern. Therefore, the concomitant administration of two agents that induce Hb F production by different mechanisms of action with different toxicity profiles, might yield an excellent combination regimen in sickle cell disease treatment and management.

We have examined the effects of didox and trimidox in combination with STZ, on the induction of hemoglobin and F-cell production in human erythroleukemia (K562) cells. In this report, we demonstrated that didox and trimidox are effective individually, and in combination with STZ possess synergistic effect in inducing hemoglobin and F-cells production in K562 cells. The optimal drug combinations (2 μM TRI + 10 μM STZ; 12 μM DI + 4 μM STZ) for induction of hemoglobin and F-cells in K562 cells closely approximated the concentration that suppresses 50% cell growth. However, as the concentra-

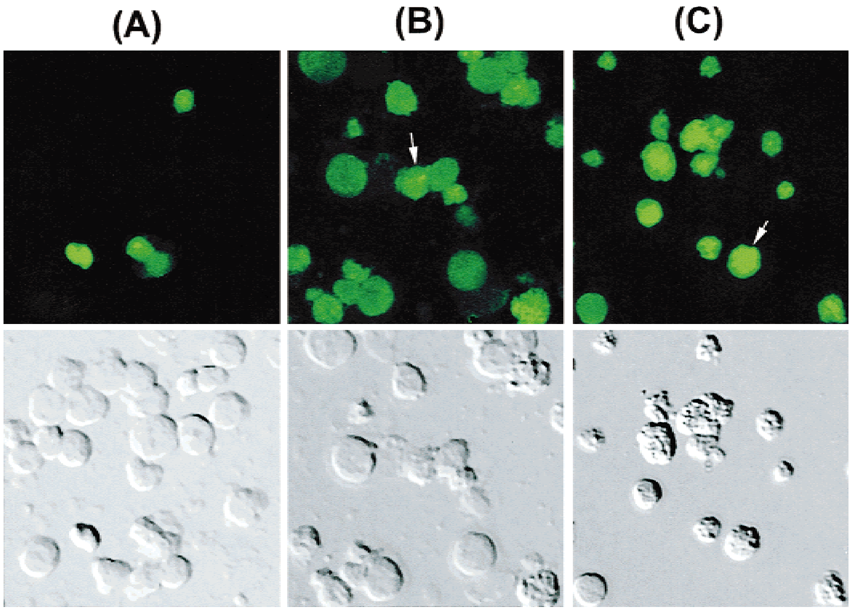

Fig. 7. Morphology of Hb F-containing cells. Upper panels represent cells obtained by fluorescence cytometry, and the lower panels represent cell morphologies as revealed by differential interference cytometry (DIC). (A) Cells in the control (untreated) sample; (B,C) cells treated with trimidox-STZ and didox-STZ, respectively. The arrow indicates representative Hb F-containing cells with high fluorescence intensity.

TABLE II. Effect of Varying Concentrations of Didox or Trimidox in Combination With STZ on Benzidine-Positive and F-Cell Production in K562 Cells†

Agents	Concentration (μM)	Benzidine-positive cells (%)	F-cells (%)	Cell viability* (%)
None	–	18	5	94 ± 2.0
Didox	23	46	20	94 ± 2.0
STZ	16	55	24	93 ± 1.0
Didox + STZ	24			
	8	24	9	40 ± 1.3
Didox + STZ	23			
	16	4	1.6	10 ± 0.5
Trimidox	7	45	15	94 ± 2.0
STZ	16	55	24	93 ± 1.0
Trimidox + STZ	3			
	15	11	4	32 ± 1.5
Trimidox + STZ	7			
	16	2.5	0.7	15.0 ± 2.0

†The K562 cells were incubated in cultured medium and supplemented with didox or trimidox and STZ. After 3 days of culture, the effect on cell viability, Hb, and F-cell levels was determined as described in Materials and Methods. Data are the means of at least three determinations, and the standard error (SEM) values were within 5%.
*Cell viability was determined by trypan blue exclusion assay.

tions of the drug combination were increased to their respective IC_{50} concentration, there was a significant cytotoxicity, and the number of benzidine-positive cells and F-cells decreased proportionately as shown in Table II.

Although the precise mechanisms by which these ribonucleotide inhibitors induce Hb F induction are still under investigation, the synergism of these novel RRIs (didox and trimidox) with STZ may be explained by their different modes of action. Streptozotocin possesses the ability to alkylate cellular DNA, effectively leading to depurination and depletion of deoxynucleotide endogenous pools. Didox and trimidox may enhance the damaging effect of STZ on DNA by preventing the synthesis of the deoxynucleotides required for repair of the DNA damaged by STZ as well as DNA synthesis required for new cells production. The inhibitory effects of these drugs on DNA synthesis may then lead to rapid recruitment of erythroblasts from progenitors with higher fetal hemoglobin program(s) during erythroid maturation [32].

The results of this study correlate for the first time the synergy of the growth inhibitory effect of didox or trimidox and STZ, with their induction of hemoglobin and F-cell production during erythroid maturation in human erythroleukemia K562 cells. Our results suggest that a combination regimen of didox or trimidox and STZ might offer an additional promising therapy for sickle cell disease if similar results could be demonstrated in vivo.

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